

Research

**Serum organochlorines and urinary porphyrin pattern in a population highly exposed to hexachlorobenzene**Jordi Sunyer<sup>\*1,2</sup>, Carmen Herrero<sup>3</sup>, Dolores Ozalla<sup>3</sup>, Maria Sala<sup>1</sup>,  
Núria Ribas-Fitó<sup>1</sup>, Joan Grimalt<sup>4</sup> and Xavier Basagaña<sup>1</sup>

Address: <sup>1</sup>Respiratory and Environmental Research Unit, Institut Municipal d'Investigació Mèdica, Barcelona, Catalonia, Spain. Dr Aiguader, 80 ; 08003 Barcelona; Spain, <sup>2</sup>Universitat Pompeu Fabra, Barcelona. Dr. Aiguader, 80; 08003 Barcelona; Spain, <sup>3</sup>Porphyria Unit. Department of Dermatology. Hospital Clinic. IDIBAPS. Faculty of Medicine, University of Barcelona. Villarroel, 170; 08036 Barcelona; Spain and <sup>4</sup>Department of Environmental Chemistry, ICER-CSIC, Barcelona. Jordi Girona Salgado, 18–26; 08034 Barcelona; Spain

E-mail: Jordi Sunyer\* - [jsunyer@imim.es](mailto:jsunyer@imim.es); Carmen Herrero - [cherrero@clinic.ub.es](mailto:cherrero@clinic.ub.es); Dolores Ozalla - [dozalla@medicina.ub.es](mailto:dozalla@medicina.ub.es); Maria Sala - [msala@ajsbadell.es](mailto:msala@ajsbadell.es); Núria Ribas-Fitó - [nribas@imim.es](mailto:nribas@imim.es); Joan Grimalt - [jgoqam@cid.csic.es](mailto:jgoqam@cid.csic.es); Xavier Basagaña - [xbasagana@imim.es](mailto:xbasagana@imim.es)

\*Corresponding author

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**Keywords:** coproporphyrin I, coproporphyrin III, hexachlorobenzene, organochlorinated compounds, porphyria, uroporphyrin

**Abstract**

**Background:** Porphyria cutanea tarda (PCT) is caused by hexachlorobenzene (HCB) in several species of laboratory mammals, but the human evidence is contradictory. In a study among adults of a population highly exposed to HCB (Flix, Catalonia, Spain), the prevalence of PCT was not increased. We aimed at analysing the association of individual urinary porphyrins with the serum concentrations of HCB and other organochlorine compounds in this highly exposed population.

**Methods:** A cross-sectional study on total porphyrins was carried out in 1994 on 604 inhabitants of the general population of Flix, older than 14 years. Of them, 241 subjects (comprising a random sample and the subgroup with the highest exposure) were included for the present study. The porphyrin profile was determined by high-pressure liquid chromatography. Serum concentrations of HCB, as well as common organochlorine compounds, were determined by gas chromatography coupled to electron capture detection.

**Results:** Coproporphyrin I (CPI) and coproporphyrin III (CPIII) were the major porphyrins excreted, while uroporphyrins I and III were only detected in 2% and 36% of the subjects respectively, and heptaporphyrins I and III in 1% and 6%, respectively. CPI and CPIII decreased with increasing HCB concentrations ( $p < 0.05$ ). This negative association was not explained by age, alcohol, smoking, or other organochlorine compounds. No association was found between uroporphyrin I and III excretion, nor heptaporphyrin excretion, and HCB. CPIII increased with smoking ( $p < 0.05$ ).

**Conclusion:** HCB exposure in this highly exposed population did not increase urinary concentrations of individual porphyrins.

The porphyrias are disorders of the haem biosynthesis in which specific patterns of overproduction of haem precursors are associated with particular clinical features. Porphyrria cutanea tarda (PCT) is one of the major potential toxic manifestations of hexachlorobenzene (HCB) in several species of laboratory mammals [1]. However, the porphyrinogenic effect of this chemical on humans has not been widely studied. The first cases of PCT induced by HCB in humans were reported in south-eastern Turkey in the late 1950s due to food poisoning in undernourished children [2–4]. Levels of HCB were not measured. In addition, there have been some case reports of workers exposed to HCB developing PCT [5], but there was no association between exposure to HCB and PCT in small studies in workers [6–8].

High atmospheric levels of HCB (mean 35  $\mu\text{g}/\text{m}^3$ ) were detected in Flix (Catalonia, Spain), a village of 5,000 inhabitants located in the vicinity of an electrochemical factory, the only industry in town [9]. Internal dose concentrations (i.e., serum) found were the highest ever reported in human general populations [10]. In a survey of 604 inhabitants only one subject had abnormally high levels of total porphyrins, compatible with PCT [11], resulting in a prevalence of PCT not higher than expected [12]. The association between individual urinary porphyrins and the internal dose of HCB was studied only in the 15 subjects with extremely high levels of HCB. Although concentrations of total porphyrins were within the range of clinical normality in this adult population [11], variations within the normality could be informative of subclinical harmful effects (i.e., functional effects). In addition, functional effects may be applied to risk assessment and prevention for its potential impact on susceptible populations [13].

Our objective was to determine the association between individual urinary porphyrin levels and serum concentrations of HCB and other organochlorine compounds in the general population of Flix.

## Material & Methods

### Study population

An epidemiological cross-sectional study was carried out on the 4,178 inhabitants of Flix older than 14 years in 1994 and details of participation and sociodemographic characteristics are described elsewhere [14]. Total porphyrins were analysed in 604 of these participants [11], a subsample of 241 of them being included in the present study. This subsample was composed of a 100 subjects previously selected for the studies of HCB biokinetics [15] consisting of those 8 subjects with the four highest and four lowest HCB levels, complemented by a random sample of 92, supplemented by a further 141 subjects who had worked in the electrochemical factory. Workers were

included because they had the highest levels of HCB in Flix [10]. In total, 177 out of the 241 subjects had worked in the electrochemical factory at some time, of whom 55 were currently employed. The individual with PCT was excluded from this analysis. All subjects were informed of the purpose of the study and signed a written consent form approved by the ethical committee at IMIM.

### Urinary porphyrin measurements

Urine samples (24 h) were collected in 2 L plastic flasks. Immediately after collection, sodium bicarbonate was added to obtain a solution of 5 g/L, and 5 ml aliquots were transferred to polypropylene tubes and stored at -20°C until analysis. All urine specimens were analysed without previous knowledge of HCB levels in serum. Urinary porphyrin excretion patterns were analysed by high performance liquid chromatography (HPLC). Briefly, 1 mL of urine sample was acidified with 50  $\mu\text{L}$  of HCl and 200  $\mu\text{L}$  of this solution was injected. The HPLC determination was performed with Waters equipment (Waters Corp. Mildford, MA, USA) (2 pumps mod. 515, an autosampler injector (mod. 717 plus) and Millennium software). The porphyrins were detected using a fluorescence detector (mod. 474), under the following conditions: excitation 405 nm, emission 618 nm, both with band widths of 18 nm. Porphyrin separation was achieved with an analytical column BDS-Hypersil (250  $\times$  4.6 mm, 5  $\mu\text{m}$  particle size) (Shandon HPLC, Cheshire, U.K) and a gradient from 100% of solvent A (10:90 acetonitrile/ammmonium acetate 1 M pH 5.16) to 95% of solvent B (10:90 acetonitrile/methanol) in 25 min. The flow rate was 1.2 mL/min [16].

### Analysis of organochlorine compounds

Organochlorine compounds in serum were analysed by gas chromatography (GC) coupled to electron capture detection and GC coupled to chemical ionisation negative-ion mass spectrometry. A Varian Star 3400 coupled to a Finnigan Mat INCOS XL was used for the analyses. All the analyses were carried out in the Department of Environmental Chemistry (CID-CSIC). Details of the methodology have been reported elsewhere [10,15]. We present results for the most prevalent compounds found in sera samples: HCB, dichlorodiphenyl dichloroethene (p,p'-DDE), and polychlorinated biphenyls (PCBs) which we present as the summation of the individual congeners 28, 52, 101, 118, 138, 153, and 180. Because the PCB congeners 138, 153 and 180 represented around 90% of total PCBs, we also provide results of these individual congeners. Detection limits for HCB and p,p'-DDE were 0.2 ng/mL, and for the individual congeners of PCBs were the following: 0.17, 0.15, 0.09, 0.11, 0.15, 0.12, and 0.10. The concentration of organochlorine compounds below the detection limit were set at half the limit of detection.

### Statistical analysis

Individual porphyrins were treated both as a dichotomous variable (detectable/non-detectable) and as continuous variable after assigning half the detectable level to represent the non-detectable values. Total porphyrins were analysed only as a continuous variable since all observations were detectable, while uroporphyrins were analysed only as a dichotomous variable since most of the observations were not-detectable. The crude association between porphyrins and the study variables (organochlorine compounds as well as confounding variables such as age, sex, alcohol, smoking and duration of the residence) was assessed with the non-parametric Wilcoxon test for linear trend, given the non-normal distribution of porphyrins. To control the association between porphyrins and organochlorine compounds for the confounding variables both linear regression models and logistic regression models were fitted. Since the data distribution of individual porphyrins was skewed, a logarithmic transformation was carried out. Organochlorine compounds were treated both as trichotomous variables and as continuous variables, given a certain linearity in the association with porphyrins. Organochlorine compounds were also logarithmically transformed (in base 10) to achieve a normal distribution. Multiple linear regression analysis was performed to examine the relationship between continuous values of porphyrins (total porphyrins, CP I, CPIII) and organochlorine levels adjusting for potential confounding variables such as creatinine in serum [14]. Association was measured with the regression coefficient. The antilogarithm of the coefficient yields the relative change in porphyrin levels for each 10-fold increase in organochlorine

compound levels. Multiple logistic regression models were fitted to estimate the adjusted association between categorical values of uroporphyrins I and III and organochlorine compounds. The measure of association was the odds ratio. Statistical significance was defined as  $p < 0.05$ . All statistical analyses were performed using Stata (StataCorp. TX, USA).

**Table 1: Characteristics of the 241 subjects**

Variable	n (%)
Male	180 (75)
Age	
< 45 years	89 (37)
45–64 years	84 (35)
> 64 years	68 (28)
Alcohol	
No	73 (30)
Occasional	61 (25)
Habitual (> 3 drinks a week)	107 (45)
Smoking	
Never	73 (30)
Occasional	11 (5)
Ex	82 (34)
Current	75 (31)
<20 cigs./day	33 (13)
≥ 20 cigs./day	42 (18)
Years living in Flix	
1–9	4 (2)
10–19	19 (8)
>20	218 (90)

**Table 2: Distribution of urinary uroporphyrins ( $\mu\text{mol/L}$ ) and serum concentrations of organochlorinated compounds ( $\text{ng/mL}$ ) (n = 241 subjects)**

	% detectable	Percentiles among detectables				
		Min	p25	p50	p75	Max
Total porphyrins	100%	13.0	63.8	98.5	157	497
Uro I	36%	2.0	6.0	10.0	16.0	37.0
Uro III	2%	1.0	1.0	1.0	8.0	8.0
Hepta I	1%	2.0	2.0	2.5	3.0	3.0
Hepta III	6%	2.0	2.8	4.0	6.8	13.0
Copro I	76%	1.0	4.0	7.0	14.0	134
Copro III	86%	2.0	10.0	21.5	37.0	154
HCB	100%	2.30	13.2	21.7	37.9	1616
DDE	97%	0.1	2.59	6.15	14.4	67.4
Total PCBs†	94%	0.2	1.89	3.78	7.02	143
CB-138	87%	0.1	0.58	1.03	1.85	35.0
CB-153	85%	0.1	0.67	1.18	2.32	40.9
CB-180	89%	0.1	0.58	1.43	2.66	63.5

Uro: uroporphyrins; Hepta: heptaporphyrins; Copro: coproporphyrins. † PCBs =  $\Sigma$  (28, 52, 101, 118, 138, 153, 180) congeners

## Results

Table 1 presents the socio-demographic and behavioural characteristics of the subjects included in this study. Not surprisingly, most of them are males given that we enriched the sample with the electrochemical workers in order to have the individuals with the highest HCB levels in the study. It is noticeable that most of them had lived in the town for more than 20 years.

Distributions of total porphyrin concentrations and individual urinary porphyrins as well as organochlorine compound levels in serum are presented in table 2. Coproporphyrin isomers I-III (CPI; CPIII) were the major porphyrins excreted. The uroporphyrin I (UPI) fraction was the third most excreted porphyrin. The heptacarboxylporphyrin isomer I (hepta I) was only detected in 1% of subjects and the heptaporphyrin isomer III (hepta III) in 6%. The hexa- and pentacarboxylporphyrin fractions were not detected. Among the organochlorine compounds, all subjects had detectable concentrations of HCB, and most of them of *p,p'*-DDE. The highest concentrations were found for HCB. The most prevalent PCB congener was CB-180.

Total porphyrins decreased with age, years of residence in Flix, and organochlorine concentrations (except CB-153) and increased with male gender and tobacco smoking, in a statistically significant way, while no association was observed with alcohol consumption (table 3). A similar pattern was observed with CPI and CPIII, except that the association with sex disappeared. However, for CPI the only statistically significant differences found were for age, years of residence, HCB and *p,p'*-DDE, but not for smoking or PCBs. For CPIII, the associations with smoking and PCBs, in addition to age, years of residence, HCB and *p,p'*-DDE, were significant. The negative association of CPI and CPIII with HCB occurred both in subjects working in the electrochemical factory (with the highest HCB levels, median of HCB = 79.2 ng/mL) and in non-workers (median HCB = 14.2 ng/mL). Workers had lower average levels of CPIII than non-workers (20.6 and 33.3  $\mu\text{mol/L}$ , respectively) and of CPI (6.8 and 11.2  $\mu\text{mol/L}$ , respectively) (all  $p < 0.05$ ). UPI and UPIII did not differ for any of the study variables (table 3), and nor did heptaporphyrin.

The negative association of HCB with total porphyrins and CPIII remained after adjusting for the confounding variables (table 4). The association between HCB and CPI was also negative but with a  $p$  value of 0.06. When HCB was treated as a trichotomous variable in the multivariate models, results were very similar to those in table 3 (where they were unadjusted), with a  $p$  value  $< 0.05$  for CPIII. In contrast, the associations of *p,p'*-DDE and PCBs disappeared in the multivariate model. HCB remained as-

sociated with total porphyrins and CPIII after adjusting for *p,p'*-DDE and PCBs. In the multivariate models, the positive associations of smoking with total porphyrins (coefficient (se) = 0.18 (0.08)), and mainly with CPIII (coefficient (se) = 0.30 (0.13)) remained, and so did the negative association of age with total porphyrins, CPI and CPIII. The adjusted association with UPI and UPIII remained non-significant.

## Discussion

In experimental porphyria in laboratory animals, intake of HCB was followed initially by a moderate increase of coproporphyrins, and later by an increase of highly carboxylated porphyrins, such as uroporphyrins and heptaporphyrins [17]. In our human population, we observed that levels of individual urinary porphyrins (uroporphyrins, heptaporphyrins, coproporphyrins) did not increase with serum HCB concentrations. Even though our results contrast with the findings to be expected from experiments in animals [17], in general, they coincide with older studies in human populations (table 5) since no increase of uroporphyrins was observed in any of these studies. In addition, in contrast with animals, coproporphyrins (mainly CPIII) were lower in subjects with higher levels of HCB which agrees with an old study in workers from a chlorinated solvents plant [8]. Furthermore, neonates of Flix also showed a decrease of coproporphyrins in relation to HCB [18], although levels of urinary porphyrins during the first 10 days of life are highly variable [19], and results difficult to interpret. The negative association with coproporphyrins was not found in a cross-sectional study in the general population in Louisiana, USA [20], where plasma concentrations of HCB were positively correlated with levels of urinary coproporphyrin, though in a weak and crude way (correlation coefficient = 0.15). However, serum concentrations of HCB among these subjects were very low in contrast with spray workers from the same location among whom there was no association between HCB and individual coproporphyrins [7]. Finally, in a study comparing 9 smelter workers exposed to HCB and to octachlorostyrene with 18 non-exposed workers, CPIII was higher among the workers [21]. Only this latter study, in addition to the studies conducted in Flix, measured the porphyrin profile using a reliable method such as the high-pressure liquid chromatography. However, the authors did not report the levels of HCB, and comparisons were not adjusted for other variables related with porphyrins such as age and smoking.

Epidemiological studies on porphyrins with other environmental agents are rare. The possible role of other halogenated compounds, such as dioxins (TCDD), in the disturbance of the porphyrin metabolism was proposed due to some case reports of PCT among workers [22]. However, this effect was mostly attributed to a concomi-

**Table 3: Association between urinary porphyrine concentrations and the study variables (age, sex, alcohol, smoking, years of residence, and organochlorinated compounds in serum).**

	TP	Copro I		Copro III		Uro I + UroIII
	Median (25%-75%)	Detectables n (%)	Median (25%-75%) †	Detectables n (%)	Median (25%-75%) †	Detectables n (%)
Age						
<45	117 (73-185)	70 (79)	7 (3-15)	85 (96)	29 (11-46)	31 (35)
45-64	89 (62-132)	62 (74)	4 (0.5-8)	67 (80)	14 (4.5-27.5)	24 (29)
>64	83 (56-126)*	51 (75)	4 (1.3-9)*	56 (82)*	12.5 (3-30)*	27 (40)
Sex						
Female	85 (62-112)	42 (69)	5 (0.5-11)	60 (98)	20 (10-31)	17 (28)
Male	107 (65-165)*	141 (78)	5 (2-11)	148 (82)*	19 (5-37)	65 (36)
Alcohol						
No	99 (62-144)	52 (71)	5 (0.5-11)	67 (92)	18 (6-35)	23 (32)
Ocasional	86 (64-143)	45 (74)	5 (0.5-10)	52 (85)	19 (7-35)	21 (34)
Habitual	103 (66-162)	86 (80)	5 (3-12)	89 (83)	20 (5-35)	38 (36)
Smoking						
Never	86 (62-124)	52 (71)	5 (0.5-10)	64 (88)	19 (6-29)	19 (26)
Ocasional + Ex	92 (60-146)	65 (70)	4 (0.5-10)	76 (82)	12 (4-35)	33 (35)
< 20 cigs./day	103 (83-159)	30 (91)	6 (4-9)	28 (85)	20 (7-39)	12 (36)
≥ 20 cigs./day	127 (81-588)*	36 (86)*	6.5 (3.5-15)	40 (95)	32 (12-46)*	18 (43)
Years living in Flix						
≤ 20 years	165 (86, 240)	21 (91)	7 (3, 19)	22 (95)	27 (17, 51)	10 (43)
> 20 years	94 (62, 143)*	162 (74)	5 (0.5, 10)*	186 (85)	19 (6, 34)*	72 (33)
HCB ng/ml (tertiles)						
<16.1	129 (81-176)	60 (77)	7 (2-15)	73 (94)	26 (8-43)	29 (37)
16.1-29.5	82 (55-126)	62 (75)	4 (0.5-10)	70 (84)	18 (5-31)	28 (34)
>29.5	92 (64-130)*	61 (76)	5 (2-8.5)*	65 (81)*	14 (5-31.5)*	25 (31)
DDE ng/ml (tertiles)						
< 3.22	124 (74-179)	62 (78)	7 (2-15)	74 (94)	26 (7-49)	31 (39)
3.22 - 9.88	105 (65-161)	60 (75)	4 (0.5-10)	64 (80)	20 (4.5-35)	22 (28)
> 9.88	82 (57-117)*	61 (74)	5 (2-8.5)*	70 (85)	12 (5-26)*	29 (35)
PCBs ng/ml (tertiles)						
< 2.08	108 (74, 166)	63 (80)	6 (2-13)	72 (91)	22 (7, 35)	21 (27)
2.08-4.89	99 (61, 172)	60 (77)	4 (2-12)	69 (88)	20 (6, 38)	30 (38)
> 4.89	87 (59, 130)*	60 (73)	4.5 (0.5-9)	67 (80)*	13.5 (4, 30)*	31 (37)
CB-138 (tertiles)						
< 0.55	108 (73-116)	63 (79)	5.5 (2.5-13)	73 (91)	20 (7.5-36.5)	19 (24)
0.55 - 1.29	99 (61-146)	63 (80)	5 (2-12)	69 (87)	21 (6-38)	36 (46)
> 1.29	89 (60-130)*	57 (70)	4 (0.5-9)	66 (80)*	13 (4-29)*	27 (33)
CB-153 (tertiles)						
< 0.61	109 (73-161)	65 (81)	6 (3-13.5)	74 (93)	21 (8.5-35)	21 (26)
0.61 - 1.36	98 (61-173)	58 (73)	4 (0.5-11)	66 (84)	20 (5-39)	30 (38)
> 1.36	87 (61-130)	60 (73)	4 (0.5-9)*	68 (83)	13 (5-32)	31 (38)
CB-180 (tertiles)						
< 0.61	110 (73-168)	64 (80)	5 (2-11.5)	72 (90)	21 (7-38.5)	22 (28)
0.61 - 1.80	94 (61-143)	59 (75)	5 (0.5-12)	73 (92)	20 (6-37)	26 (33)
> 1.80	90 (60-141)*	60 (73)	4.5 (0.5-10)	63 (77)*	14 (3-29)*	34 (41)

TP: total porphyrins; CPI, CPIII: coproporphyrins I, III; UPI, UPIII: uroporphyrins I, III. \* p < 0.05 using the extension of the non-parametric Wilcoxon test for linear trend † non-detectable values were set at the median value between 0 and the detectable level

**Table 4: Adjusted association between urinary porphyrin levels and serum concentrations of organochlorinated compounds (n = 241).**

Organochlorinated compound	TP	Copro I	Copro III
Coefficient (se) <sup>†</sup>			
Single pollutant model			
HCB	-0.265 (0.078)*	-0.321 (0.171)	-0.395 (0.187)*
DDE	-0.024 (0.049)	0.048 (0.115)	-0.051 (0.123)
PCBs	-0.005 (0.048)	-0.039 (0.111)	-0.019 (0.120)
Multipollutant model <sup>††</sup>			
HCB	-0.281 (0.079)*	-0.315 (0.174)	-0.420 (0.190)*
DDE	-0.056 (0.053)	0.039 (0.127)	-0.092 (0.137)
PCBs	0.005 (0.052)	-0.059 (0.121)	-0.011 (0.131)

TP: Total porphyrins. CPI and CIII: coproporphyrins, I and III, respectively. † The coefficient gives the relative change in porphyrin levels for a 10-fold concentration increase in the organochlorinated compound, adjusted for age, smoking, alcohol, years of residence in Flix, and creatinine in serum. †† Adjusted for age, smoking, alcohol and years of residence in Flix, as well as for the other organochlorinated compounds in the table. \* p < 0.05

**Table 5: Studies on urinary porphyrins in human populations exposed to moderate levels of hexachlorobenzene (HCB).**

Author year/country (ref)	N	Period	Serum-HCB (range in ng/mL)	Porphyrins UP	CP	Comments
<b>WORKERS</b>						
Morley 1973/Australia [6]	54	1950's-60's	NA	NS	NS	only 1 worker with high UP
Burns 1974/USA [7]	20	1973-74	<1-310	NS	NS	-
Currier 1980/USA [8]	50	1974-7	3-1121	NS	↓	↓ CP if HCB > 200
Selden 1999/Sweden [21]	27	1980's	NA(9E/18NE)	NS	↑	↑ CIII in E vs NE
<b>GENERAL POPULATION</b>						
<b>NEONATES</b>						
Ozalla 2002/Spain [18]	68	1997-99	0.4-21	NS	↓	↓ CPI and CIII
<b>ADULTS</b>						
Burns 1975/USA [20]	120	1972	0-23	NS	↑	-
This study/Spain	242	1994	2-1616	NS	↓	↓ CPI and CIII

NA: not available. NS: no statistically significant difference. UP: uroporphyrins. CP, CPI and CIII: coproporphyrins total, I and III. E/NE: number of subjects exposed/non-exposed

tant exposure to HCB [22]. Recent epidemiological studies in human populations did not find any association of TCDD exposure with regard to urinary uroporphyrin or coproporphyrin levels [23,24]. A negative association between arsenic concentration in urine and CIII was found in 36 individuals from the general population in an area of Mexico with high levels of arsenic in drinking water compared with 31 non-exposed subjects [25]. In contrast with studies on HCB, a concomitant increase in uroporphyrins occurred. Also, in a crosssectional study carried out in a convention of dentists, the 38 dentists with levels of

total mercury higher than 20 µg/l had higher levels (twice) of urinary coproporphyrins than the 23 dentists with no detectable levels of mercury [26]. Mechanisms of action for these compounds, however, could be of a different nature than those related with HCB.

In accordance with our previous observation of an association of passive smoking with CIII in neonates [18], active smoking is associated with an increase of porphyrins in the present study. In patients with intermittent porphyria, smoking was associated with induction of repeated

acute attacks of porphyria [27]. This finding agrees with a possible indirect effect of smoke through a cytochrome P450 induction, since some P450 isoenzymes such as P4501A2s, which is affected by smoking, are involved in disturbances of porphyrin metabolism [28]. Recently it has been suggested that HCB should be classified as a dioxin-like compound that binds the Aryl hydrocarbon (Ah) receptor inducing hepatic cytochrome p4502B activity [29]. Why smoking is associated with an increase of CPIII while HCB was associated with a decrease of CPI and CPIII, without modifying uroporphyrin levels, is an intriguing problem to solve.

Overall, the epidemiological studies in human populations have to be considered with caution given their size, the methodology used to measure the porphyrin profile, the control of the effect of variables such as age and smoking and the difficulties in disentangling the individual pollutant effects from the pollution mixtures. A conclusion from such review is the lack of a common procedure in environmental studies of porphyria. In addition, porphyrin increases have been related with the multiple chemical sensitivity syndrome (MCS) [13]. However, there is currently no convincing evidence that MCS syndrome affects the haem synthesis [13]. Our findings confirm that it is premature and speculative to relate porphyrin increases with current environmental exposures.

The present results do not seem affected by a selection bias given that similar findings were observed both in the random and the enriched sample. Cross-sectional bias also seems improbable since exposure to HCB could not be determined by knowledge of levels of uroporphyrins and viceversa. A confounding effect due to an unmeasured variable seems also unlikely. Positive serology against virus C was only found in 4 of the subjects, and renal dysfunction was detected in a very small proportion [14]. A final explanation could be the presence of an error due to misclassification of uroporphyrins or HCB levels, non-differential, that led to the dilution of the association between HCB and uroporphyrins. Nevertheless, an error of this type should also have affected the association with smoking, while a positive association with smoking was found. In addition, in the present study porphyrin patterns and HCB levels were determined with the best current technology. These facts suggest that the effect of this misclassification error if any must be small and unlikely to explain the complete lack of porphyrin increase according to the categories of HCB.

The present study is the largest ever conducted, used a reliable method of measuring porphyrin patterns and incorporated analysis of cofactors. In addition, the population under study is specifically exposed to high levels of HCB

(serum concentrations were around 20 times higher than in unexposed Europeans of the same age [10], and levels of other pollutants such as PCB, DDE or dioxins were within the range of most populations [9]).

## Conclusion

The present results suggest that current levels of HCB are not associated with an increase of the individual uroporphyrins, and if anything are associated with a decrease of the coproporphyrins, which challenges the concept of a porphyrinogenic effect of environmental pollutants at current levels of exposure.

## Abbreviations

PCT Porphyria Cutanea Tarda

HCB Hexachlorobenzene

CPI Coproporphyrin I

CPIII Coproporphyrin III

*p,p'*-DDE Dichlorodiphenyl Dichloroethene

PCB's Polychlorinated Biphenyls

UPI Uroporphyrin I

Hepta I Heptacarboxylporphyrin isomer I

Hepta III Heptaporphyrin isomer III

MCS Multiple Chemical Sensitivity

UPIII Uroporphyrin III

## Authors' contribution

JS was the principal investigator and drafted the manuscript, CH and DO carried out the porphyrin analysis and actively participated in the drafting. MS and NRF participated in the design of the study, the contact with participants and the freeze chain. JO did the organochlorine analyses and XB the statistical analysis.

## Competing Interests

None

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